United States Department of Agriculture

Marketing and Regulatory Programs

Animal and Plant Health Inspection Service

Veterinary Services

National Veterinary Services Laboratories

National Animal Health Laboratory Network

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Assay Performance Characteristics Summary Sheet

Assay: Tetracore/United States Department of Agriculture Agricultural Research Service Classical swine fever virus (CSFV) real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assay

Disease: Classical swine fever (CSF)

Agent: Classical swine fever virus (CSFV, Select Agent)

Type of Assay: rRT-PCR assay

Purpose of Assay: Screening [National Animal Health Laboratory Network (NAHLN)], Confirmatory [National Veterinary Services Laboratories (NVSL) reference laboratories]

Background Information: A single-tube Classical swine fever virus (CSFV) real-time reverse-transcriptase polymerase chain reaction (rRT-PCR) assay (1,2) was designed for use on the Cepheid SmartCycler®. The CSFV assay was originally developed in a "vitrified" format designed to be performed on the Cepheid SmartCycler®. Subsequently, a wet chemistry version of this assay was shown to be equivalent to the "vitrified" version when performed on the Cepheid SmartCycler®.

Platform(s): Cepheid SmartCycler®

Chemistry(ies): TaqMan® Ez rTth Kit

Sample Type: Tissue grown virus, Tonsil, Nasal Swabs.

Species: Swine

Performance Characteristics: Analytical performance characteristics were determined using titered CSF viruses obtained from the Foreign Animal Disease Diagnostic Laboratory (FADDL) reference collection.

Cepheid SmartCycler®: "Vitrified Assay"

Cepheid SmartCycler® "Vitrified Assay"	
Analytical Sensitivity	< 1-2 $TCID_{50}$/ml
Analytical Specificity	100%

Amplification Efficiency: 78%

Repeatability:

Serial variations with a known status sample (Intra-Assay Variability): Intra-assay variation (standard deviation of the mean Ct value within the run) tested over the linear range of the vitrified assay on the Cepheid SmartCycler®. Intra-assay variation was tested using clinical samples and tissue culture grown virus. Results showed that the Ct values all fell within 0.73-1.69 standard deviations from the mean.

Interassay Variation: Run variations with a known status sample: A well characterized tissue culture grown virus (CSFV Brescia) was tested in multiple runs by the same user. Interassay variation fell between 0.93-1.99 standard deviations from the mean.

Diagnostic Performance Characteristics:
Diagnostic performance characteristics were determined using nasal swab samples obtained from the EU CSF Reference Laboratory in Hannover, Germany and 9 farms in the Dominican Republic. Negative reference samples (nasal swabs) for this study were collected from swine in Texas and Colombia. Samples were tested on the vitrified assay on the Cepheid SmartCycler®.

Negative reference Samples (Texas and Colombia) n=1315; Performance Estimates shown with 95% CI.

Cepheid SmartCycler®: "Vitrified" Assay—Negative Reference Samples	
Diagnostic Specificity	99.45 (98.86-100)

Reference Samples (Dominican Republic) n=446; Performance Estimates shown with 95% CI.

Cepheid SmartCycler®: "Vitrified" Assay—Dominican Republic Samples	
Diagnostic Sensitivity	92.21 (86.22-98.19)
Diagnostic Specificity	98.92 (97.86-99.97)

Reference Samples (EU Reference Laboratory, Hannover) n=185; Performance Estimates shown with 95% CI.

Cepheid SmartCycler®: "Vitrified" Assay—EU Reference Laboratory (Hannover) Samples	
Diagnostic Sensitivity	98.92 (97.43-100)

References:

1. Risatti G, Holinka L, Lu Z, Kutish G, Callahan JD, Nelson WM, Brea Tio E, and Borca MV. 2005. Diagnostic evaluation of a real-time reverse transcriptase PCR assay for detection of classical swine fever virus. *J Clin Microbiol* 43:468-471.
2. Risatti GR, Callahan JD, Nelson WM, and Borca MV. 2003. Rapid detection of classical swine fever virus by a portable real-time reverse transcriptase PCR assay. *J Clin Microbiol* 41:500-505.